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EFFECT OF NUCLEOTIDES ON RAT LIVER & SKELETAL MUSCLE MITOCHONDRIA NON-PHOSPHORYLATING RESPIRATION & MEMBRANE POTENTIAL. M. Jekabsons & B.A. Horwitz, Neurobiol., Physiol., & Behavior, Univ. Calif, Davis CA 95616.

Non-phosphorylating respiration (VO_2) is primarily controlled by proton (H^+) leak across the inner mitochondrial membrane (IMM). Recently discovered genes whose predicted amino acid sequences place them in the same family as uncoupling protein-1 (UCP-1) may be the physical basis for this leak. To determine if the leak in isolated liver (L) and skeletal muscle (SM) mitochondria is regulated similarly to UCP-1, we measured effects of nucleotides on non-phosphorylating VO_2 with a Clark electrode and IMM voltage (IMMV) with the voltage sensitive dye JC-1 (0.47µM). Mitochondria were incubated at 37C in a KCl based reaction buffer (pH 6.9) containing 3 µg/mL oligomycin, 5 µM rotenone, and 5 mM succinate. Nucleotide effects on VO_2 and IMMV are summarized in the table; percents are peak changes from the succinate induced value.

	GTP	ATP	AMP	CTP	CMP
L VO2	-20%	-97%* (11.0)	-13%	-90%* (14.6)	+75%* (4.43)
L IMMV	ND	-88%* (12.5)	-27%*	-84%* (14.1)	-17%*
SM VO2	-23%*	-99%* (11.2)	-34%*	-79%* (16.3)	+15%* (4.16)
SM IMMV	ND	-96%* (11.1)	-36%*	-86%* (15.6)	-20%*

*$p<0.05$ for effect of the nucleotide (0.8-21mM) by one way ANOVA; IC_{50} (ATP, CTP) and EC_{50} (CMP) values in mM in (); ND=not determined.

We conclude that VO_2 inhibition by ATP, CTP, and AMP reflects respiratory chain rather than leak inhibition (i.e., IMMV doesn't increase). In contrast, CMP stimulation of VO_2 and inhibition of IMMV suggest possible CMP regulation of H^+ leak in L and SM mitochondria. Regulation of this leak thus differs from that mediated by UCP-1. [NIH DK-32907, T32-HL-07682]